

APPENDIX B

Data Supplement for CHOP Disclosure #13, October 30th, 2001

Experiments have been carried out demonstrating proof of concept and feasibility for reverse gene therapy. A long QT syndrome mutation, Q9E-MiRP1 (Figure 1), was cloned into a bicistronic expression vector (CMV promoter), with a FLAG peptide sequence at the C-terminus, and a neomycin resistance region. The wild-type MiRP1 sequence was cloned into the identical vector for comparisons. Q9E has been reported to cause the long QT syndrome, and life-threatening arrhythmias following clarithromycin exposure (Cell 97:175, 1999). Therefore, when used locally in site specific reverse gene therapy, Q9E is quiescent, unless clarithromycin is administered, hypothetically resulting in slowing of the conduction in rapid re-entrant arrhythmia circuits. Thus, in practice a gene delivery implant in the heart loaded with vectors for Q9E-MiRP1 could result in overexpression of this channel in a desired region, that then later could respond selectively (the rest of the heart would not respond) to clarithromycin for clinically indicated anti-arrhythmia therapy.

Figure 2: Illustrates successful transfection using lipofection with G418 sorting of HEK293 cells, demonstrating vector expression (via green fluorescent protein), and membrane localization of either the wild-type channel or Q9E, as shown by FLAG-rhodamine immunohistochemistry per Laser confocal microscopy. Patch clamp data with current vs. voltage clamp plots are shown on the right side of the Figure, demonstrating dramatically diminished I_{Kr} currents with 1.5mM clarithromycin administration, thereby documenting the antibiotic selective effect.

Figure 3: Demonstrates successful transfection with GFP & MiRP1 expression (either wild type or Q9E) in mesenchymal stem cells. Laser confocal microscopy (Rhodamine-immunohistochemistry-anti-FLAG) documents cell membrane localization of either the wild-type or Q9E MiRP channel. These data demonstrate that this approach could be used to modify stem cells, which could then be used in actual cardiac regeneration transplants, to provide regions of the myocardium which would be responsive to this reverse gene therapy approach, re. selective regional responsiveness to clarithromycin as above.

Figure 4: Results of a five animal study demonstrating Q9E-MiRP1 (or wild-type vector) pig atrial myocardial gene expression one week following vector administration using DNA antibody micelles (50µg DNA per 100 microliter injection site) showing GFP positive cells, as documented with immunohistochemistry. H& E studies demonstrate minimal to no inflammation.

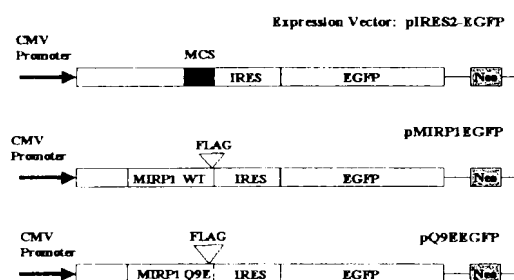
Figure 5: Morphometry studies of the pig right atrial samples (per Figure 4) indicated that 15% of cardiac myocytes were transfected with only 50µg of DNA in the injected region, with the same level of transfection per morphometry studies for either the wild-type or Q9E mutations. Laser confocal microscopy of frozen sections of atrial myocardium (Rhodamine-immunohistochemistry-anti-FLAG) demonstrates cell membrane localization of either the wild-type or Q9E MiRP channel.

CHOP 13 Disclosure, Data Supplement

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Figure 1

Expression Constructs



MIRP1 Point Mutations

			TM Domain
Rat MIRP1:	1	MTTLANLTQILEDAFKKVFITMDSWRRNTTAEQQALQARVDAENFYTVILYLAVMIGMF	60
Human MIRP1	1	M+TL+M TQILEDF+++FITMD+MR+MTTAEQ+ALQA+VDAENFYTVILYLAVMIGMF	60
		Q9E	M54T I57T
		T8A	
Rat MIRP1:	61	AFIVVAILVSTVSKRRREHSQDFYHQYIVEDWQQKYRSQILHLEDSKATIMENLGATGFT	120
Human MIRP1:	61	+FI+VAILVSTVSKRRREHS DQYHQYIVEDWQ+KY+SQIL+LE+SKATIMEN+GA GF	120
			A116V
Rat MIRP1:	121	VSP 123	
		+BP	
Human MIRP1	121	MSP 123	

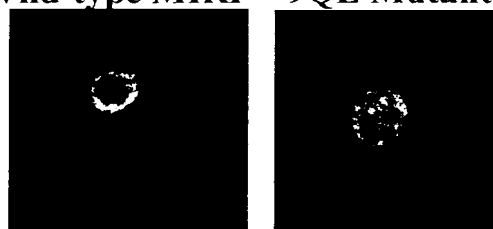
MIRP1 Mutants	Drugs induced arrhythmia (increase channel blockage)	References
Q9E	clarithromycin	Cell 97:175,1999
M54T	procainamide	PNAS 97:10613,2000
I57T	oxatamide	PNAS <i>ibid.</i>
T8A	sulfamethoxazole	PNAS <i>ibid.</i>
A116V	quinidine	PNAS <i>ibid.</i>

Figure 2

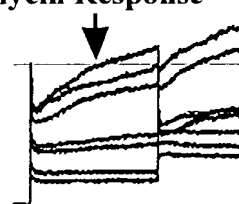
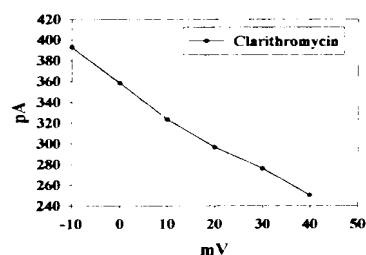
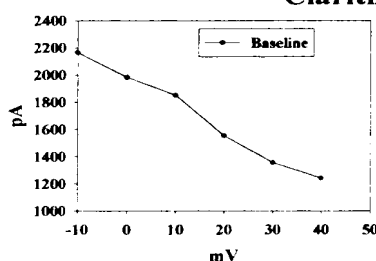
9QE Cell Electrophysiology Data Showing Clarithromycin Response

HEK 293 Cells

Wild-type MIRP 9QE Mutant



FITC=GFP,
Rhodamine=anti-FLAG-[MIRP]



Arrows indicate end of
activation pulse

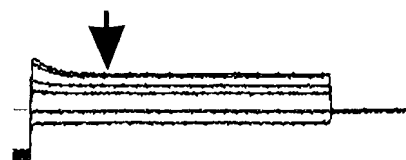
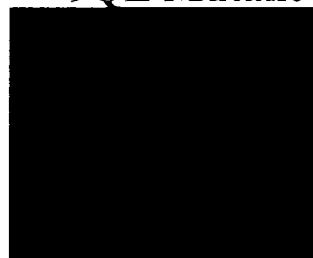
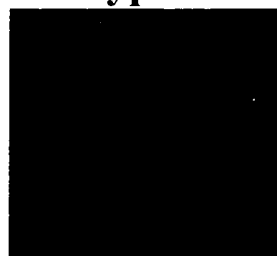


Figure 3

Rat Mesenchymal Stem Cells
Wild-type MIRP 9QE Mutant



FITC=GFP

Rhodamine=anti-FLAG-[MIRP]

Figure 4: Pig Myocardial Results

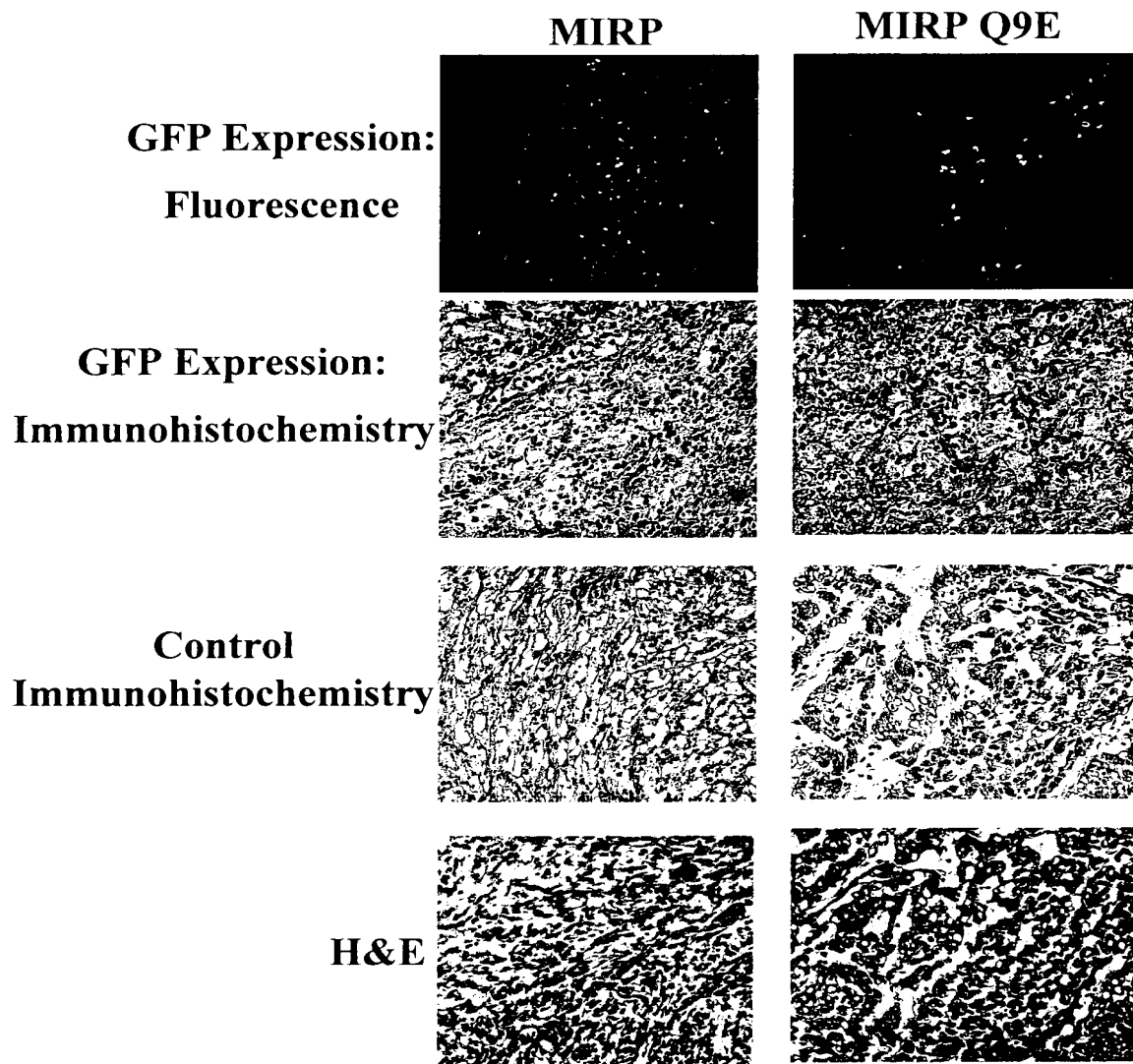
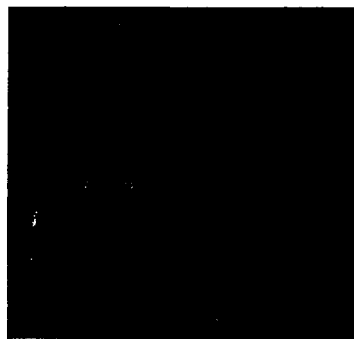


Figure 5: Pig Myocardial Results

MIRP

MIRP Q9E



Blue=DAPI

FITC=GFP

Rhodamine=anti-FLAG-[MIRP]

